

Taxol: Discovery, Mechanism and Cancer Treatment

Susan Band Horwitz, Ph.D.

Jung-Min Lee, M.D.

No Disclosures

Learning Objectives

1. History of the development of Taxol.
2. Importance of understanding the mechanism of action of antitumor drugs.
3. Role of natural products in the development of antitumor drugs.

“Taxol[®], Tubulin and Tumors: A Story of Drug Development”

Taxus brevifolia

Western Yew

Demystifying Medicine -NIH January 28, 2020



Appreciation of the Complexity of Nature

The Role of Endophytes in the Production of Taxol

Endophytes are microorganisms, in the case of Taxol, fungi that exist in plant tissue for at least part of their life cycle. During this time there is a symbiotic relationship between the tree and the fungi.

Molecules that Changed the World

Aspirin[®]

Willow Tree

Quinine

Cinchona Tree

Morphine

Opium Poppy

Steroids and the Pill

Mexican Yam

Penicillin

Mold

Mevicor[®], Zocor[®], Lipitor[®]

Fungus

Vinblastine, Vincristine

Periwinkle Plant

Taxol[®]

Yew Tree



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

NATIONAL CANCER INSTITUTE

April 21, 1977

Dr. Susan Horwitz
Assistant Professor
Department of Pharmacology
Albert Einstein College of
Medicine of Yeshiva University
1300 Morris Park Avenue
Bronx, New York 10461

Dear Susan:

At a recent Decision Network meeting, NSC-125973 (Taxol) was approved for further study. We have some information about it (folder enclosed) and believe that it may be a protein synthesis inhibitor. Would you please study this compound in your systems.

The compound is quite insoluble in aqueous vehicle, but DMA and DMSO can be used effectively.

Sincerely,

David

David Abraham, Ph. D.
Investigational Drug Branch
Cancer Therapy Evaluation Program
Division of Cancer Treatment
National Cancer Institute

Taxus brevifolia



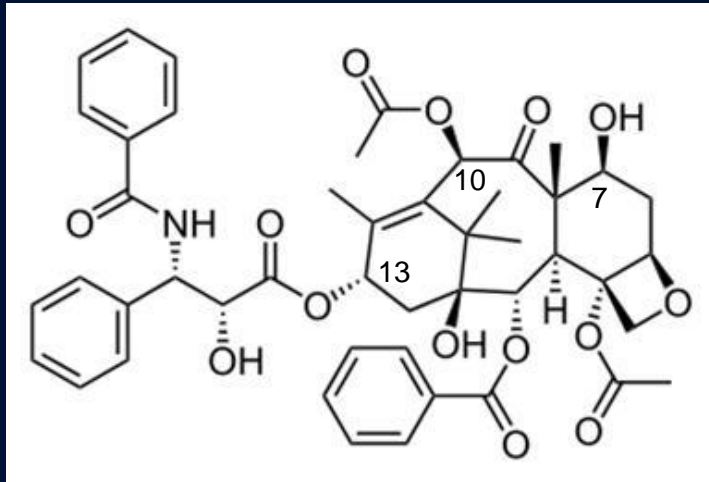
Harry Potter and the Sorcerer's Stone

J.K. Rowling

Mr. Ollivander touched the lightning scar on Harry's forehead with a long, white finger.

"I'm sorry to say I sold the wand that did it," he said softly. "Thirteen-and-a-half-inches. **Yew**. Powerful wand, very powerful, and in the wrong hands... well, if I'd known what that wand was going out into the world to do..."

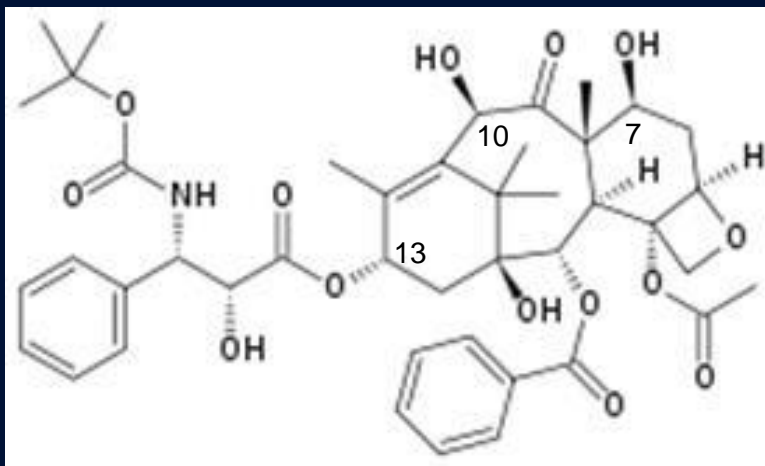
Paclitaxel (Taxol®)



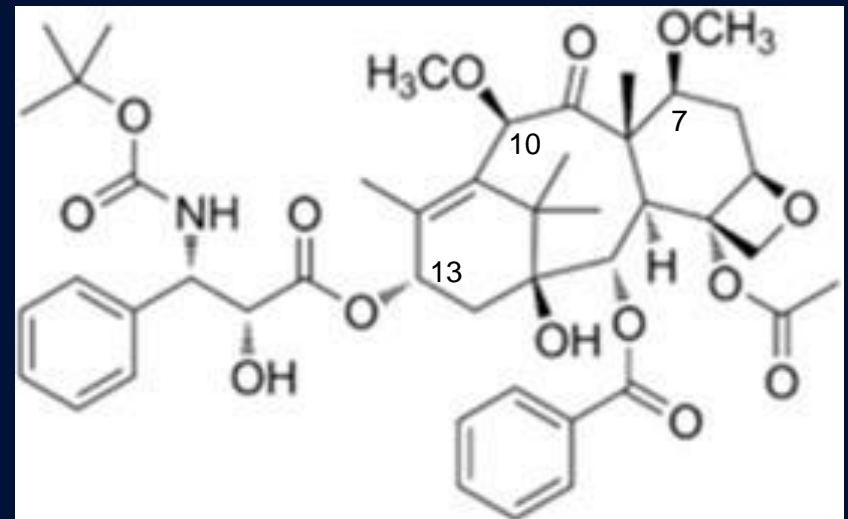
Abraxane®

Protein-bound particles for injectable suspension (albumin-bound)

Docetaxel (Taxotere®)



Cabazitaxel (Jevtana®)



The Cytoskeleton

3 families of proteins, assemble to form 3 types of filaments. Dynamic, allowing cells to respond rapidly.

1. ACTIN FILAMENTS

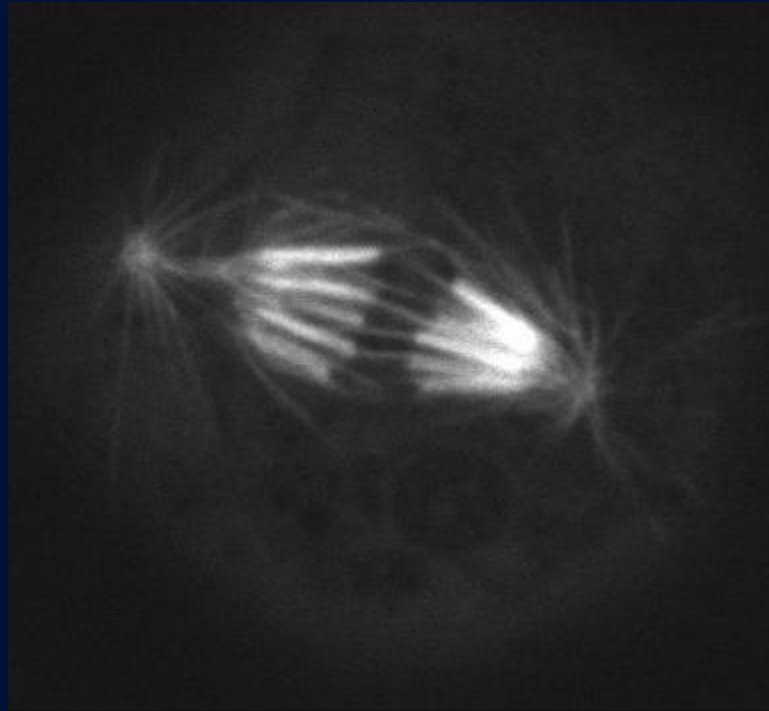
2. INTERMEDIATE FILAMENTS

3. MICROTUBULES

} accessory proteins

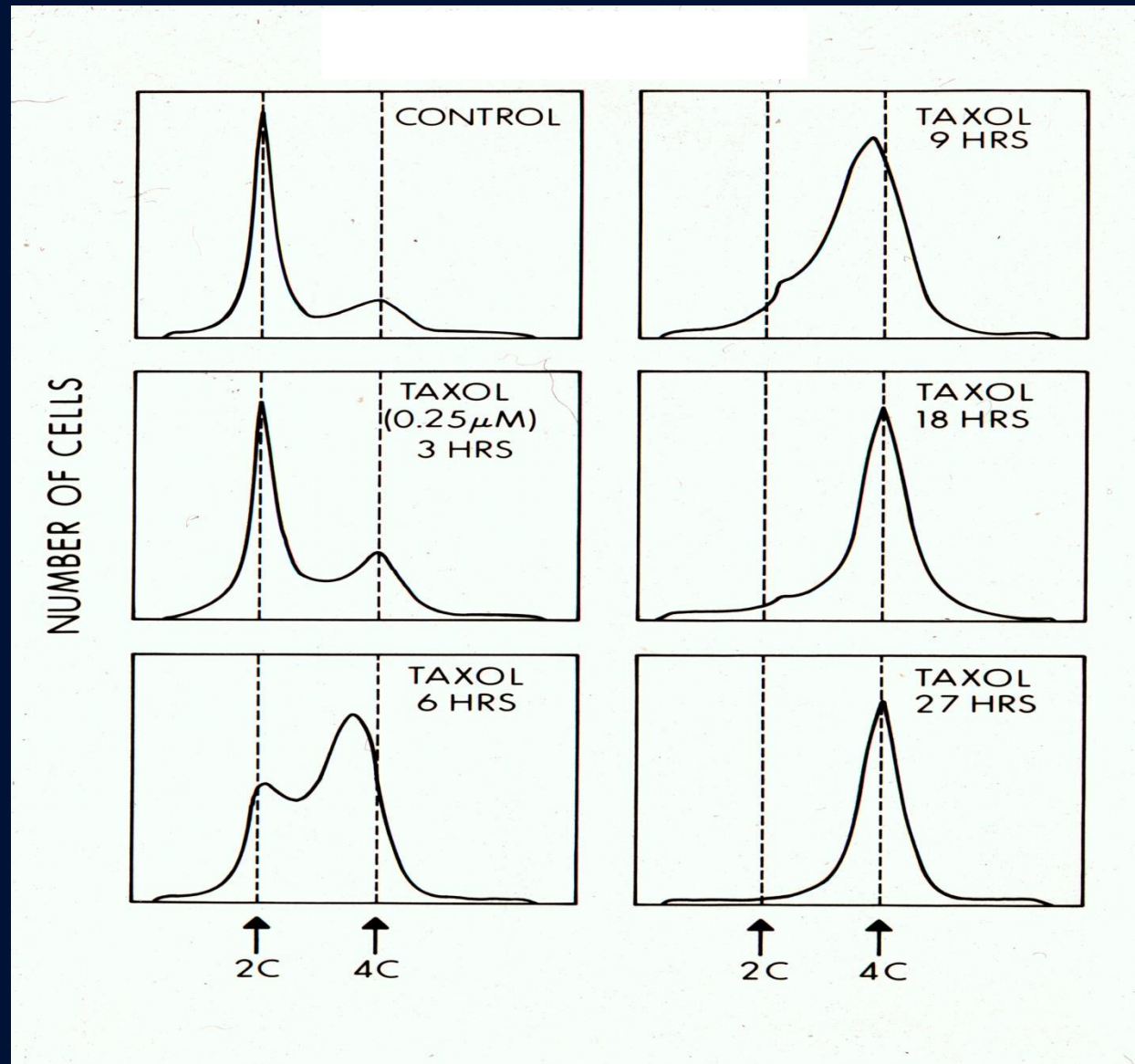
Mitosis, traffic of organelles, support membranes, sperm swim, white blood cells crawl, muscles contract, axons and dendrites extend in nerve cells, cells change shape.

GFP-Tubulin in Mitosis

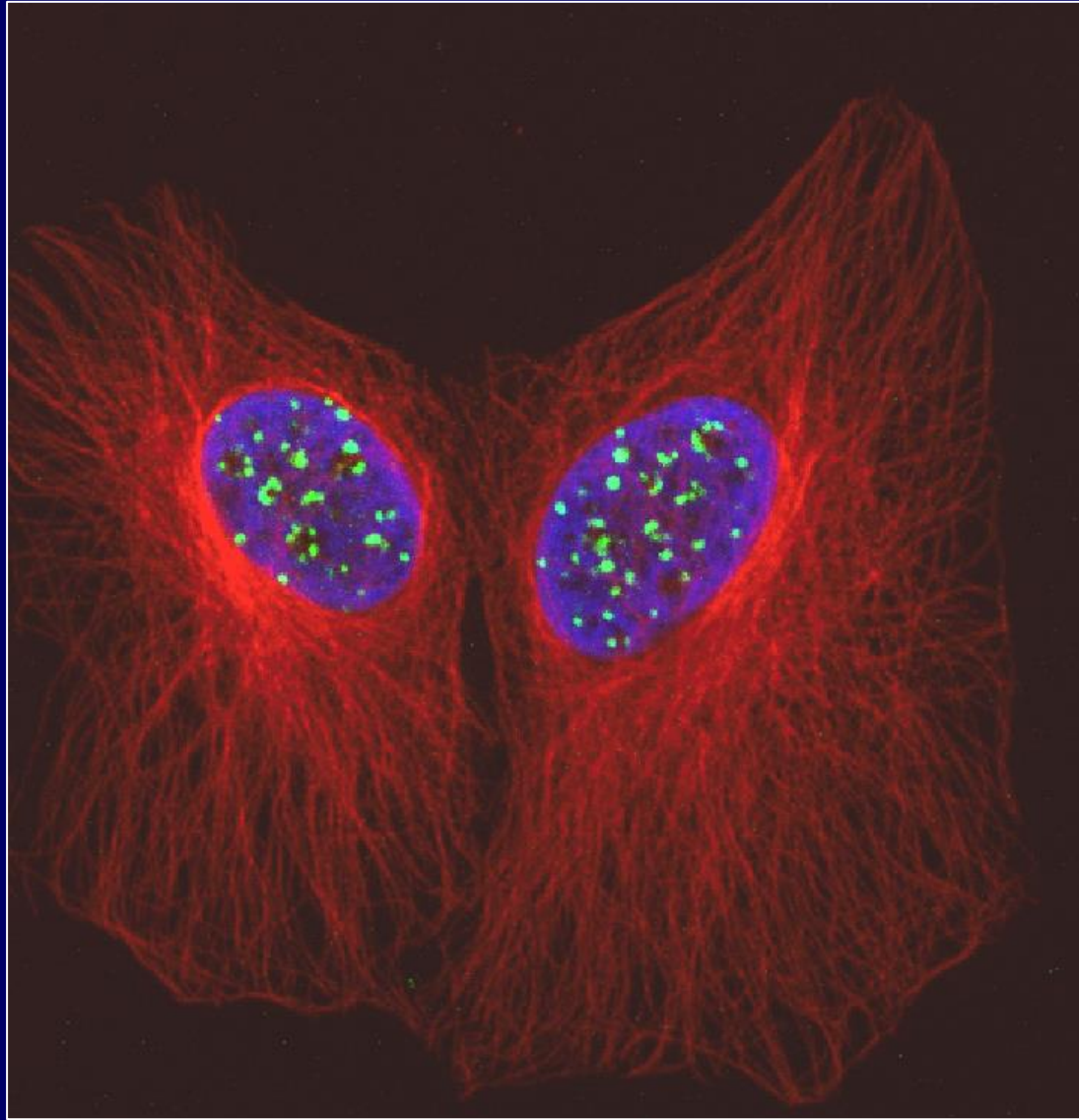


David Sharp

Taxol Blocks HeLa Cells in Mitosis

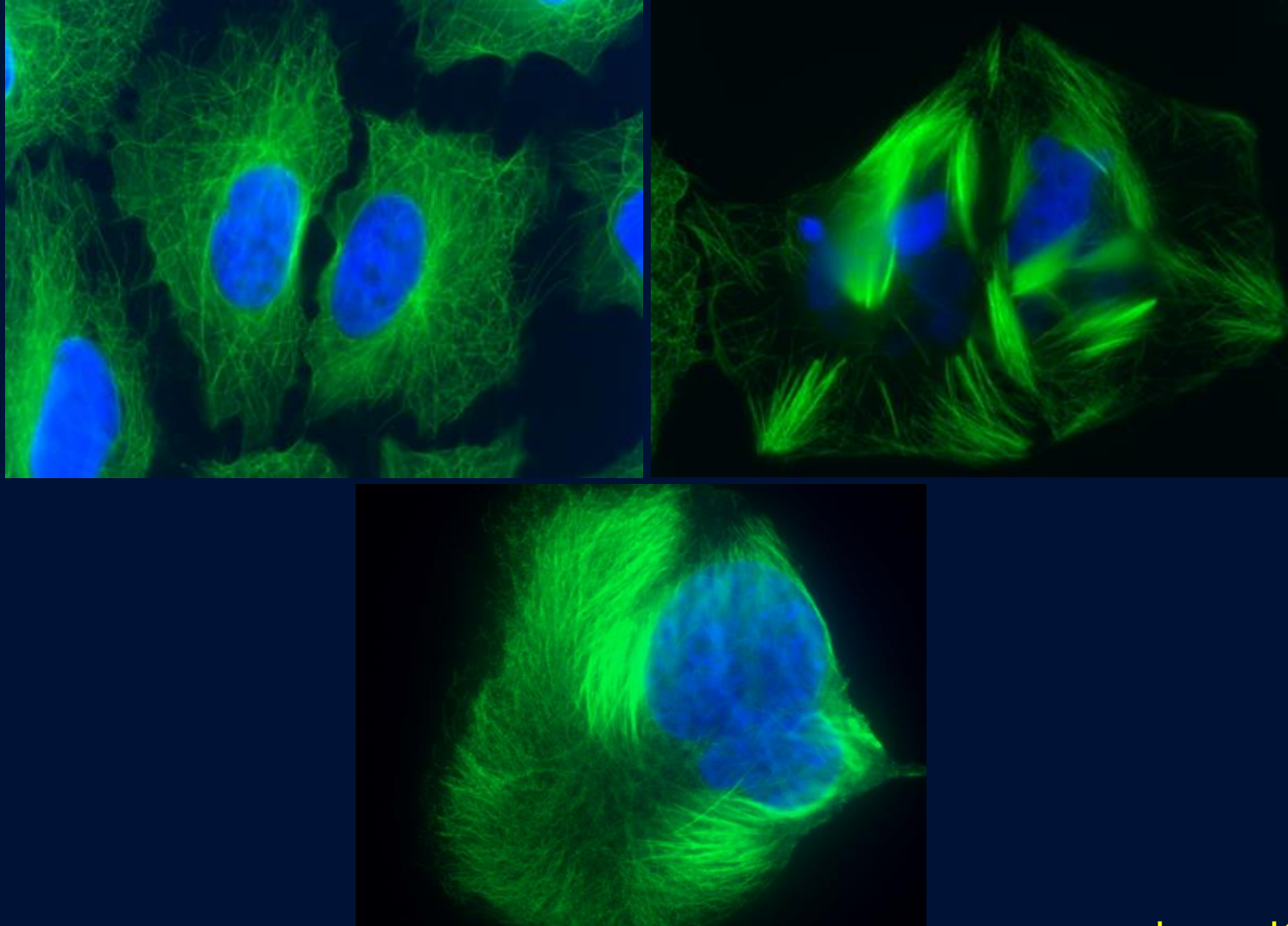


Interphase Cells

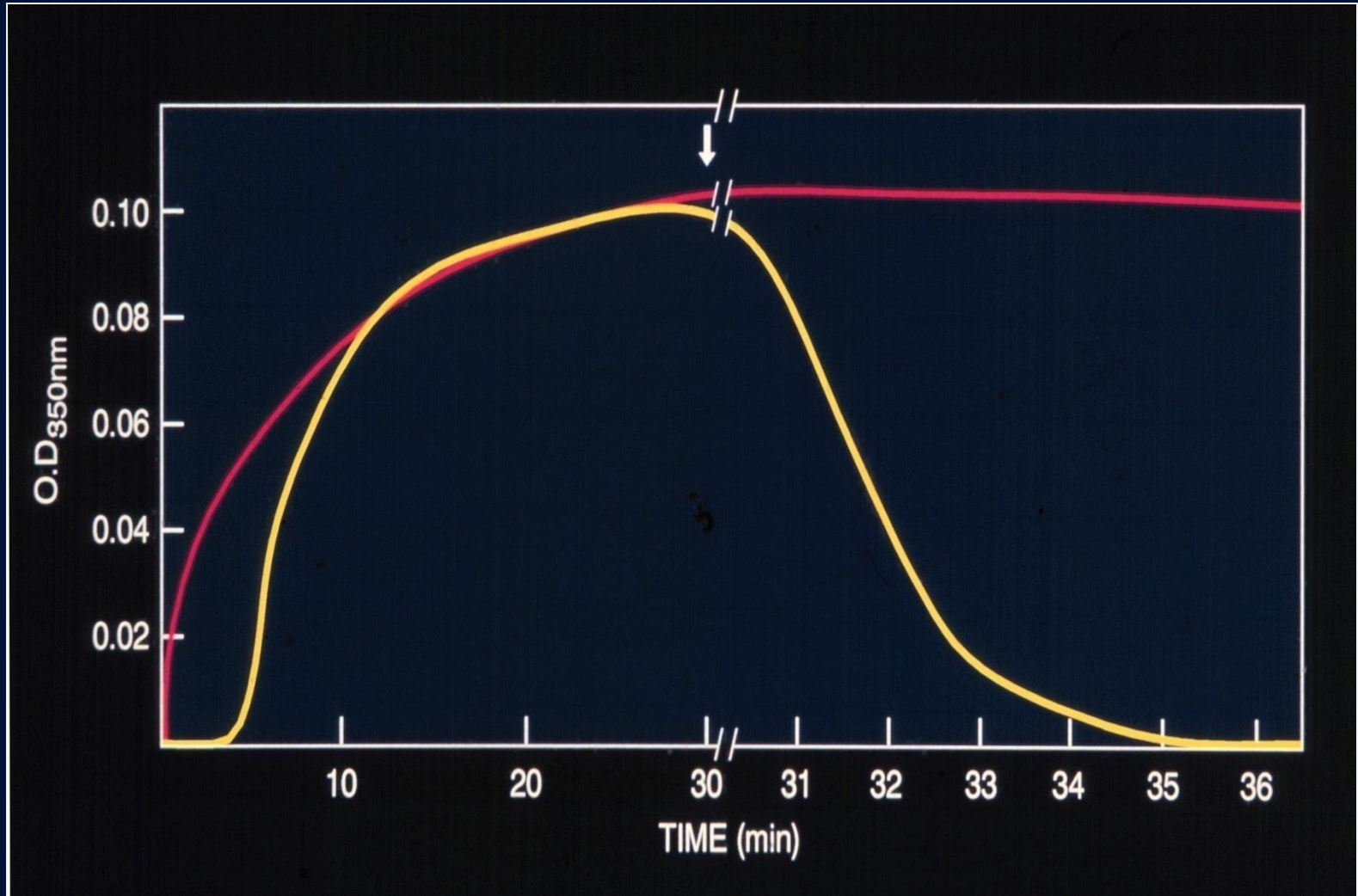


Mary Ann Jordan

Taxol induces microtubule bundling

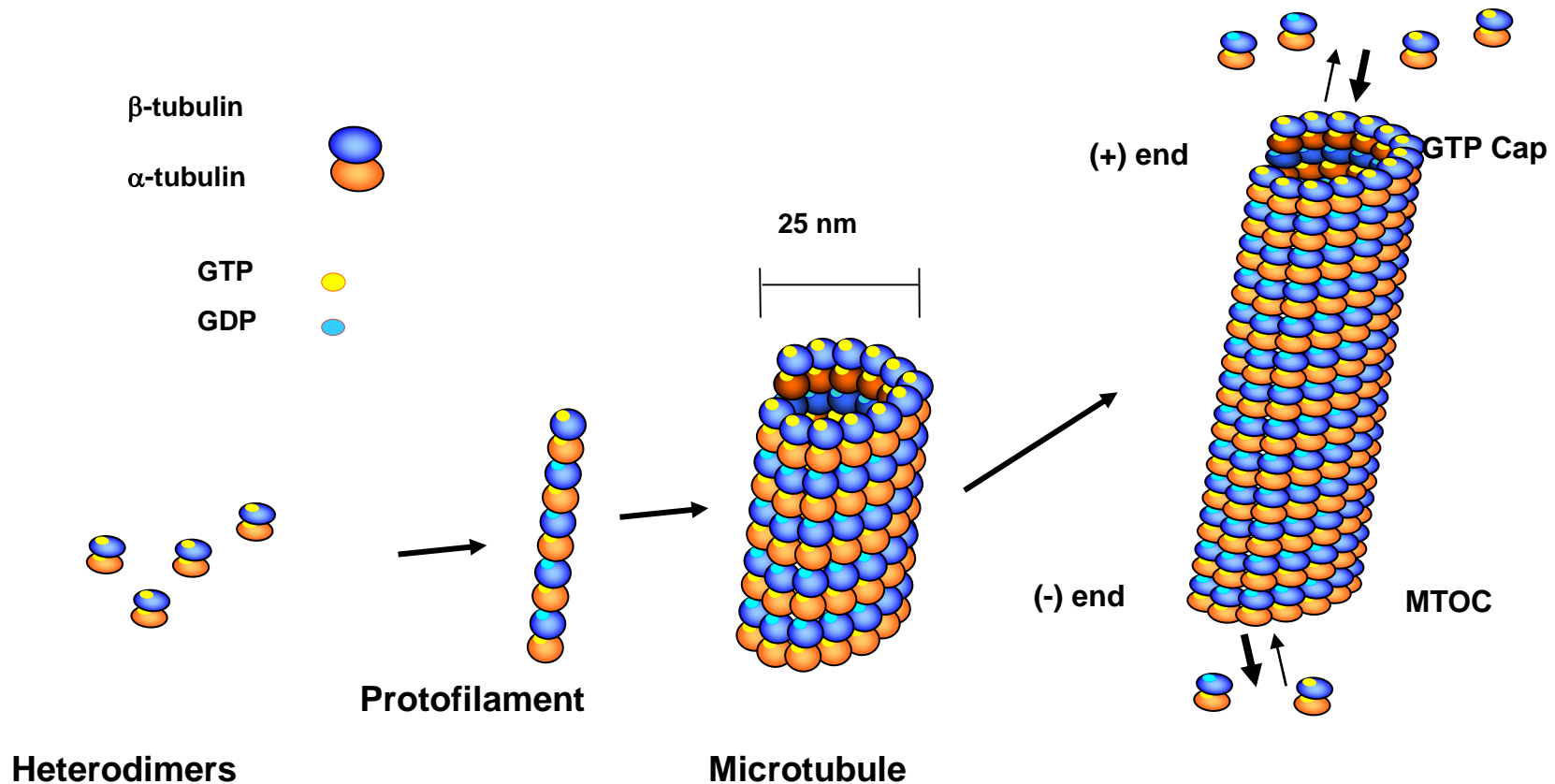


Taxol Enhances *In vitro* Tubulin Polymerization and Microtubule Stabilization



Schiff et. al., 1979, Nature

Microtubule Structure and Dynamics



- Microtubules (MTs) are highly dynamic and switch stochastically from shrinking (catastrophe) to growing (rescue) both in *vivo* and in *vitro*—DYNAMIC INSTABILITY
- Numerous isotypes (7 α , and 8 β) differ mainly at their acidic, negatively charged termini that interact with various intrinsic proteins (MAPs, kinesins, stathmin, etc.)

ALBERT EINSTEIN COLLEGE OF MEDICINE
OF YESHIVA UNIVERSITY

1300 MORRIS PARK AVENUE. BRONX, N.Y. 10461. CABLE EINCOLLMED, N.Y.

DEPARTMENT OF MOLECULAR PHARMACOLOGY

PHONE: (212) 430-2000

August 9, 1978

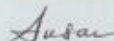
Dr. John Douros
Drug Development Branch
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

Dear John:

As I mentioned to you in Hawaii, my laboratory has become very interested in the mechanism of action of taxol. We have been working intensely with this drug during the past year and find that it is extremely cytotoxic to cells growing in culture. Although we have not completely defined its site of action, we know that it is quite different from any other drug that we have previously studied and we plan to pursue its activity. In order to do this, we need radio-actively labeled taxol. Monroe Wall and M.C. Wani isolated taxol and would certainly be the most knowledgeable concerning the preparation of labeled drug. I would, of course, include them in any publications that might develop from material they prepared. I would appreciate it if you could bring this problem to their attention. We would also like to test the two major products isolated from taxol after mild base-catalyzed methanolysis, $C_{17}H_{17}NO_4$ and $C_{29}H_{36}O_{10}$, as described in JACS 93:9, 1971.

I enjoyed talking with you in Hawaii. Thank you very much for your help.

Sincerely,



Susan B. Horwitz, Ph.D.
Associate Professor

SBH:mr



DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
BETHESDA, MARYLAND 20014

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NATIONAL CANCER INSTITUTE

August 22, 1978

Dr. Monroe E. Wall
Research Triangle Institute
P. O. Box 12194
Research Triangle Park, NC 27709

Dear Monroe:

Can you help this poor girl (enclosed letter). Please send me a quote
on these radiolabeled materials and I will buy them from you.

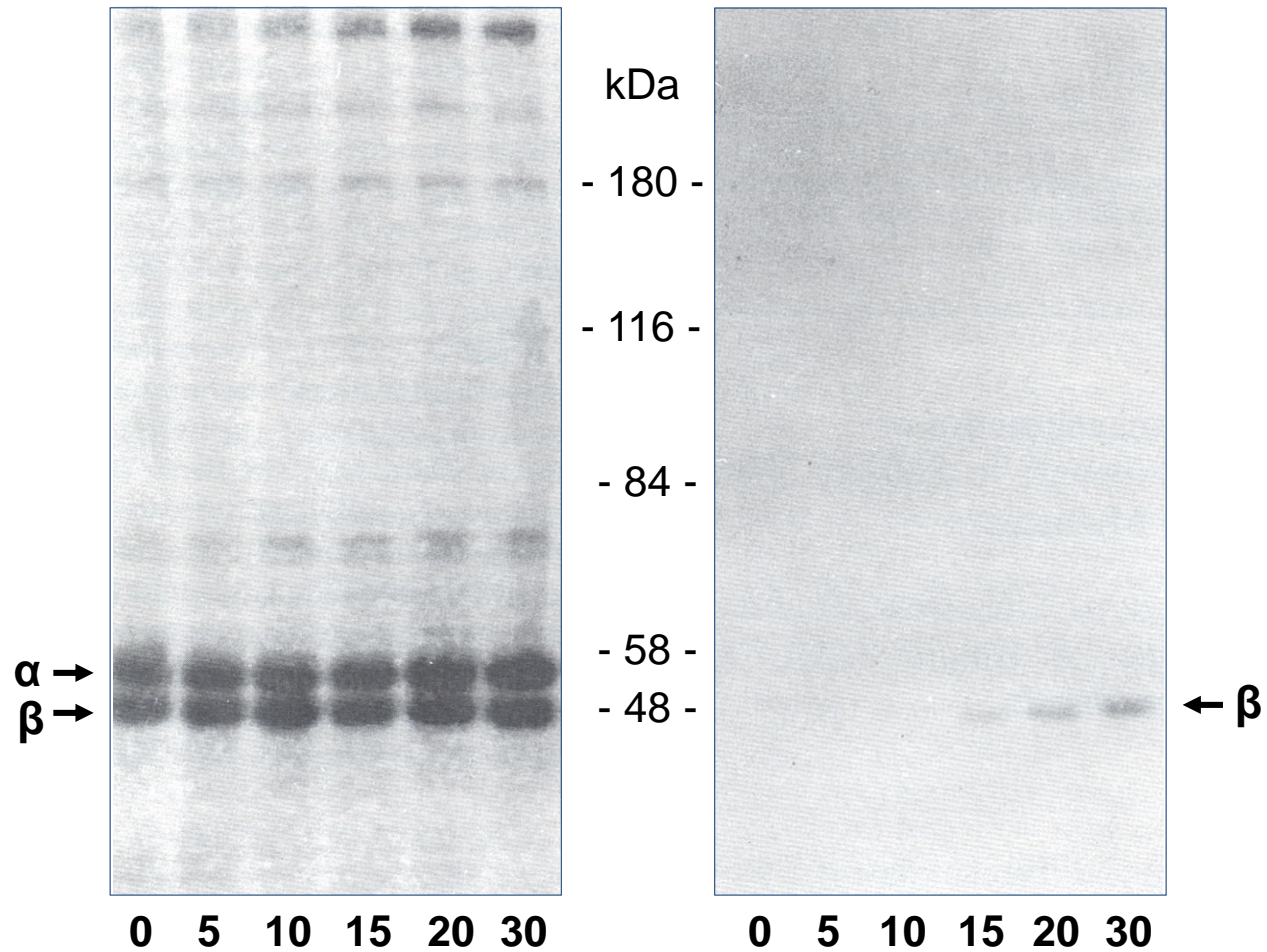
Sincerely,

John D. Douros, Ph.D.
Chief, Natural Products Branch
Developmental Therapeutics Program
Division of Cancer Treatment, NCI

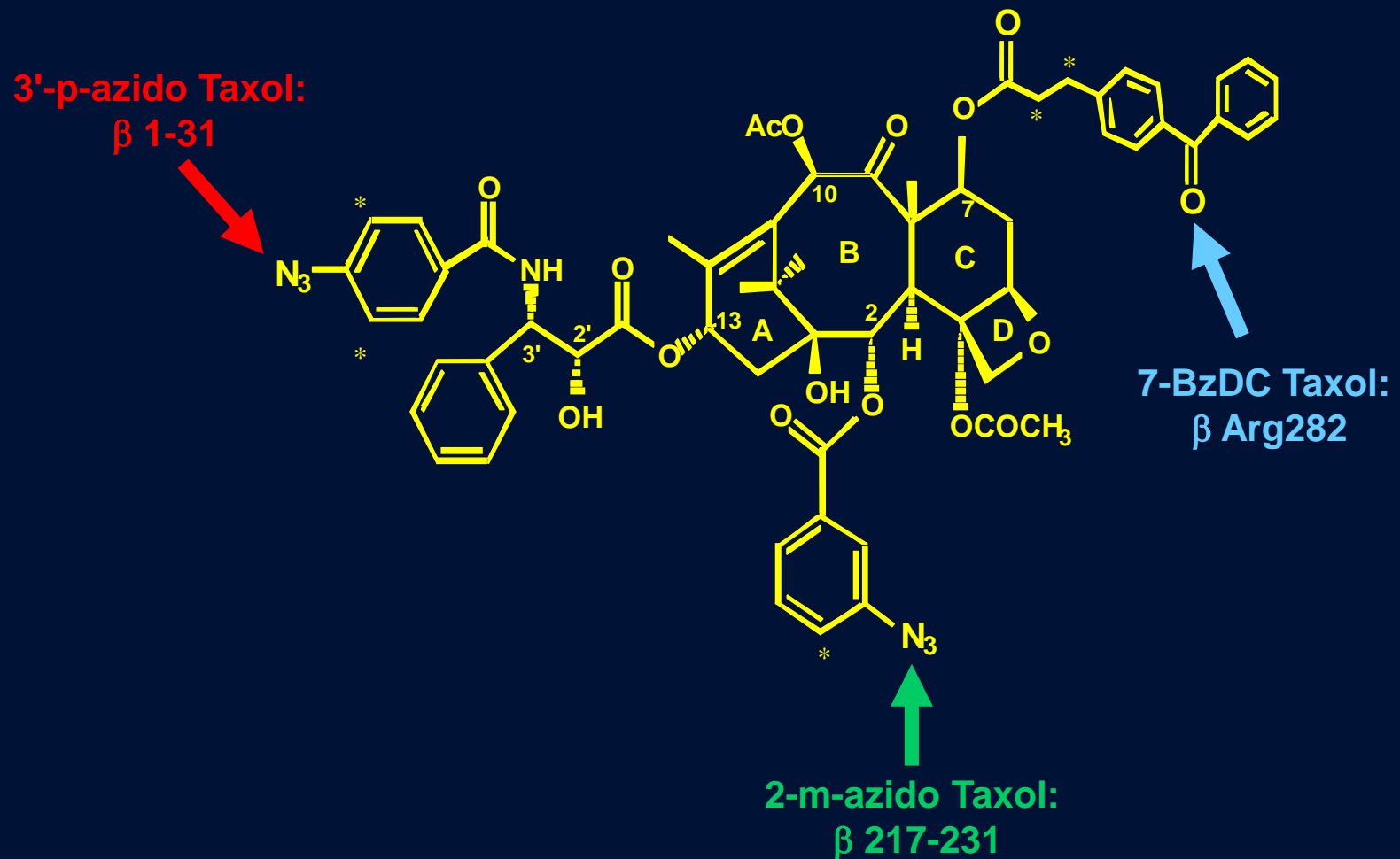
enclosure

M. W. Wani
M. K. Kessler for reply

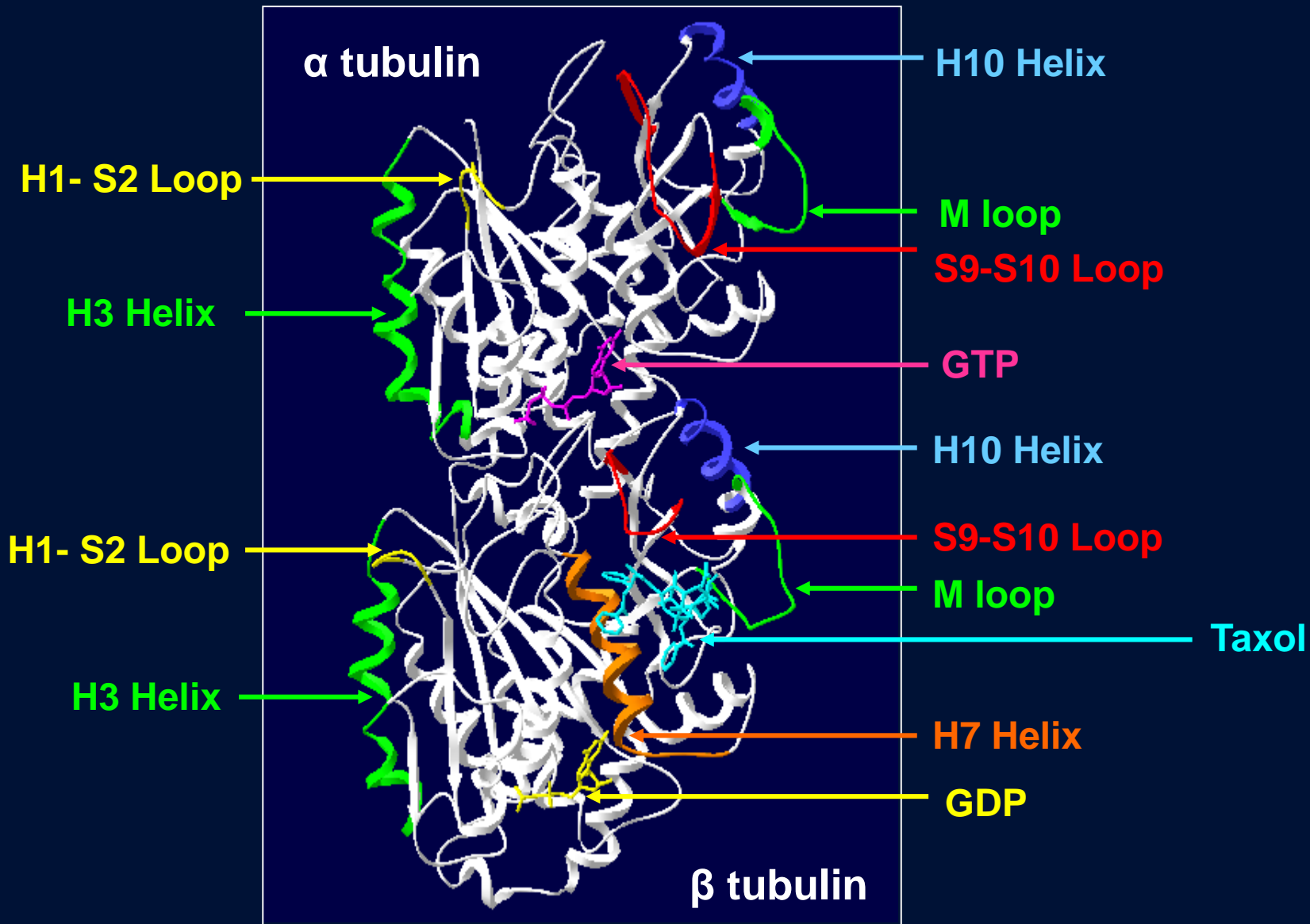
Taxol Photolabels β -Tubulin



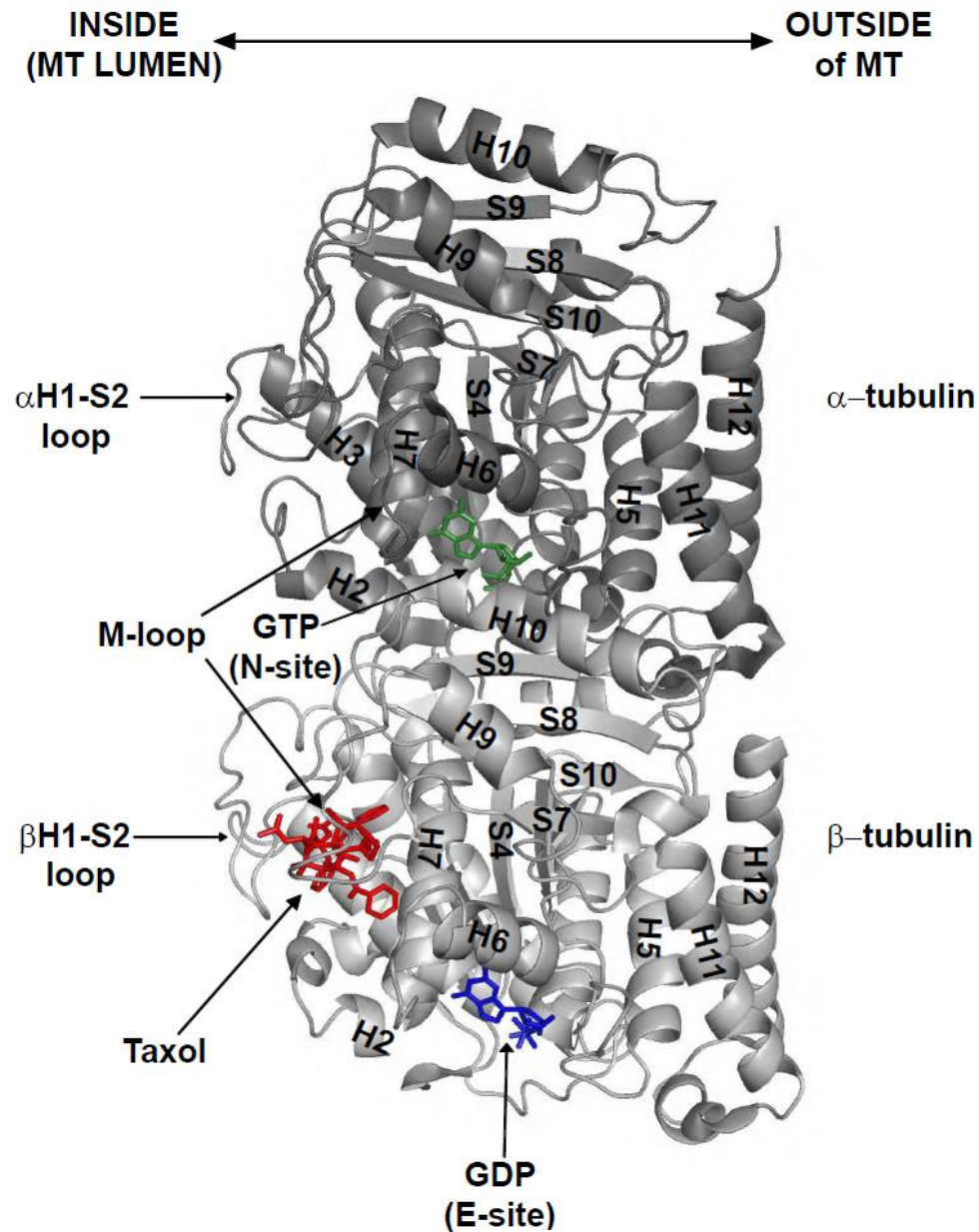
Photoaffinity Labeling Results



Taxol in the three-dimensional model of $\alpha\beta$ -tubulin dimer



Structure of the $\alpha\beta$ -tubulin heterodimer



Based on Nogales, et al, 1998

Taxol Chronology I

- 1958** Collaboration NCI/USDA
- 1962** NCI/USDA random plant collections
Extract of bark from *Taxus brevifolia*, cytotoxic to KB cells
- 1971** Taxol isolated and structure published
cytotoxic to L-1210, P-388, P-1543 leukemias, WM-256
carcinosarcoma
- 1975** Excellent activity against murine B16 melanoma
- 1977** Selected for clinical development by NCI
- 1978** Good activity against some human xenografts,
MX-1 mammary tumor
- 1979** Unique mechanism of action described

Taxol Chronology II

- | | |
|------|---|
| 1980 | Preclinical toxicology
Aqueous insolubility (Cremophor); Neutropenia |
| 1982 | Approved by NCI for IND application |
| 1983 | Clinical Trials
Hypersensitivity reactions; Scarcity of taxol |
| 1988 | Clinical activity
Advanced drug-refractory ovarian carcinoma
Metastatic breast carcinoma
Evaluation in a variety of malignancies
Combination chemotherapy / radiation / G-CSF |
| 1991 | January 23 rd . Cooperative Research & Development Agreement (CRADA) NCI and BMS |
| 1992 | July 22 nd . NDA by BMS for ovarian cancer |
| 1992 | December 29 th . FDA approval, refractory ovarian cancer |
| 1994 | FDA approval, breast carcinoma |
| 1999 | FDA approval, non-small cell lung carcinoma |
| 2013 | FDA approval, Abraxane® in combination with Gemcitabine for metastatic adenocarcinoma of the pancreas |

Names for hi-jacking

“Taxol is a trademark now, but Bristol-Myers Squibb should return it to the research community”.

Nature, Feb 2nd, 1995

Trademark

Taxol[®]

Taxotere[®]

Generic

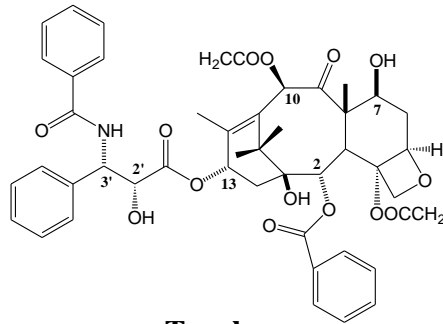
Paclitaxel

Docetaxel

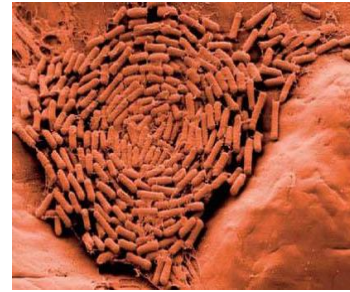
Microtubule Stabilizing Agents (MSAs)



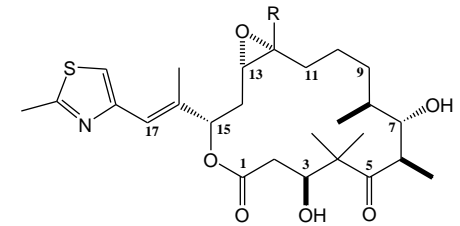
Pacific Yew



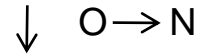
Taxol



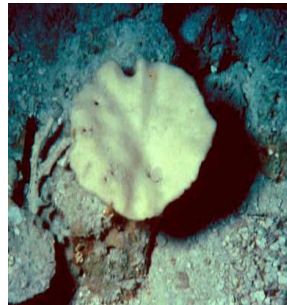
Sorangium Cellulosum



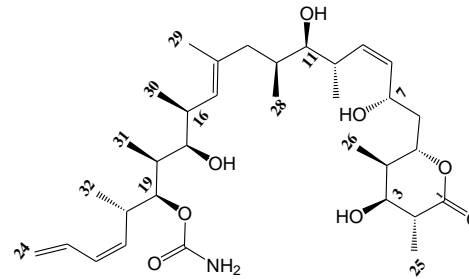
**Epothilone A (R=H)
Epothilone B (R=Me)**



Ixabepilone (IXEMPRA™)



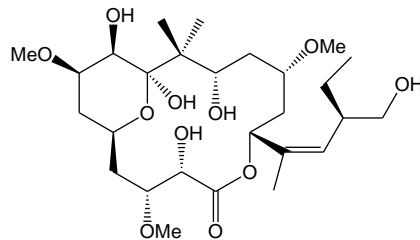
Discodermia dissoluta



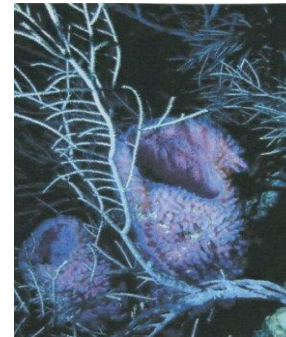
(+)-Discodermolide



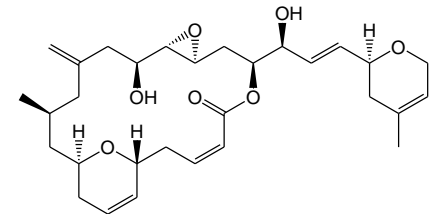
Mycale hentscheli



(+)-Peloruside A



Fasciospongia rimosa



(-)-Laulimalide

Diversity of microtubule function is reflected in a diversity of tubulin isotypes expressed within a cell.

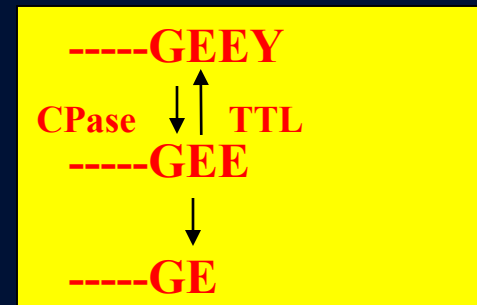
- Multiple α - and β -tubulin genes that are differentially expressed in cell/tissues.
- The C-terminal peptides of all isotypes are highly divergent and are highly acidic.
- The C-terminal peptides are located on the surface of the microtubule and are the interaction domains for a number of regulatory/motor proteins.

β I	411	AESNMNDLVS	EYQQYQDATA	EEEEDFGEEA	EEEEA
β II	411	-----	-----	D-QGE-E--E	G-DEA
β III	411	-----	-----	---GEMY-DD	---SEAQGPK
β IVa	411	-----	-----	-QG-FEE-AE	--V-
β IVb	411	-----	-----	---GE-E---	---VA
β V	411	-----	-----	NDG-EAF-DE	---IDG
β VI	411	--N-IH----	----F---K-	VL---EEVTE	-A-MEPEDKG H

“Isotype-defining region”

Tubulin isotypes also undergo extensive posttranslational modifications.

- Polyglutamylation (n=1-20, α - and β -tubulins)
- Polyglycylation (n = 1-40, α - and β -tubulins)
- Reversible tyrosination and removal of penultimate Glu to form $\Delta 2$ -tubulin in α -tubulin.



- Phosphorylation of β III (Ser_{441/444})
- Acetylation of Lys₄₀ (α -tubulin)
- Palmitoylation of Cys₃₇₆ (yeast α -tubulin)

(With the exception of acetylation and palmitoylation, all PTMs occur within the isotype-defining domain.)

β-Tubulin Isootypes

Tubulin Class	Gene Name	Protein Name	Chromosome Location	Organ Expression	Cellular Expression
Class I	TUBB	βI	6q21.33	Constitutive	All Cells
Class II	TUBB2A	βIIa	6p25.2	Brain, Nerves, Muscles	Some Neurons
Class II	TUBB2B	βIIb	6p25.2	Brain, Muscles, Tonsils	Some Neurons
Class III	TUBB3	βIII	16q24.3	Brain, Testis, Colon	Neurons Sertoli Cells Epithelial Cells
Class IV	TUBB4A	βIVa	19p13.3	Brain	Neurons Glia
Class IV	TUBB4B	βIVb	9q34.3	Most Organs	Testis Ciliated Cells
Class V	TUBB6	βV	18p11.21	Tissue Specific	Muscle Endothelial Secretory Cells
Class VI	TUBB1	βVI	20q13.32	Blood, Marrow, Spleen	Erythroid Cells Platelets

Distribution of β V-Tubulin in Normal Tissues by Immunohistochemistry

Tissue	Intensity	Tissue	Intensity
Skin	Sebaceous glands +++	Prostate	Glandular cells ++ (variable)
	Squamous epithelium + (variable)		Basal cells -
	Basal cells +	Mammary gland	Luminal cells -
Muscle	Smooth muscle +++		Lactating secretory cells ++
	Skeletal muscle ++		Myoepithelial cells +++
	Cardiac Muscle ++	Ovary	Surface epithelium -
Blood vessel	Endothelial cells +++		Stromal cells +
	smooth muscle ++	Fallopian tube	Epithelium -
GI tract ^a	Epithelium -		Pneumocytes -
Liver	Hepatocytes -		Respiratory epithelium -
	Bile ducts ++	Thyroid	Follicular cells - to ++ (variable)
Pancreas	Ducts +		Cortex and medulla -
	Islets ++	Salivary Gland	Acini + (variable)
	Acini -		Ducts ++
Kidney	Renal tubules - to ++ (variable)	Nerve	Neuronal cells -
	glomeruli ++		
Testis	Seminiferous tubules -		
	Mature germ cells -		
	Immature germ cells ++		
	Sertoli cells -		

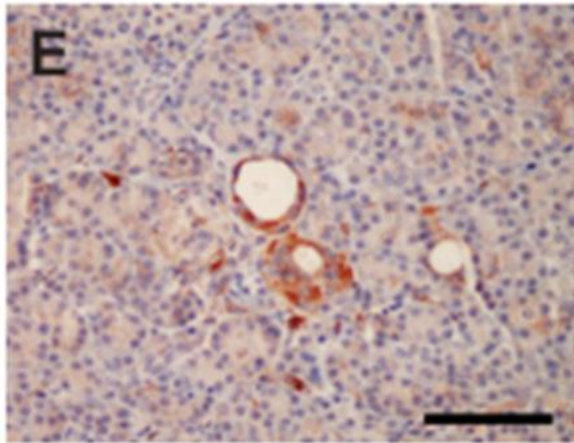


- Tissues with secretory function showing β V-tubulin expression

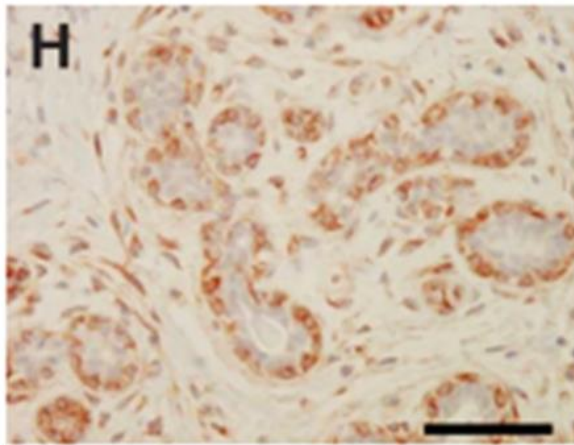
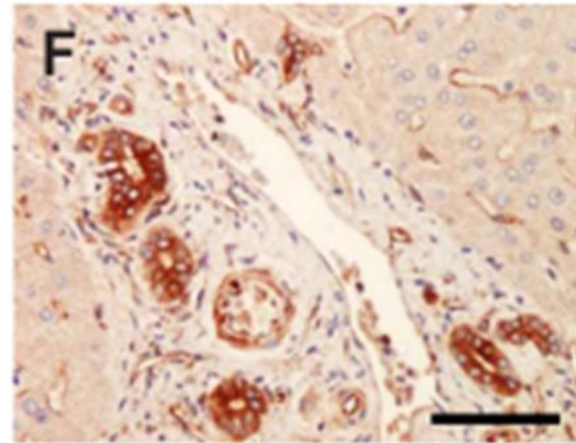
^a Stomach, Small Intestine, Appendix, Colon, Gall Bladder

β V-tubulin in Secretion

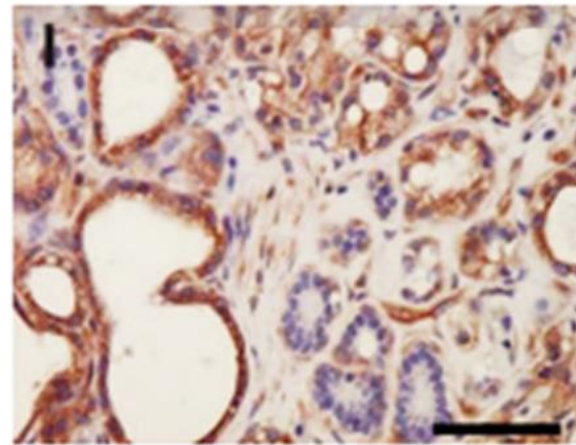
Pancreatic Duct



Bile Duct



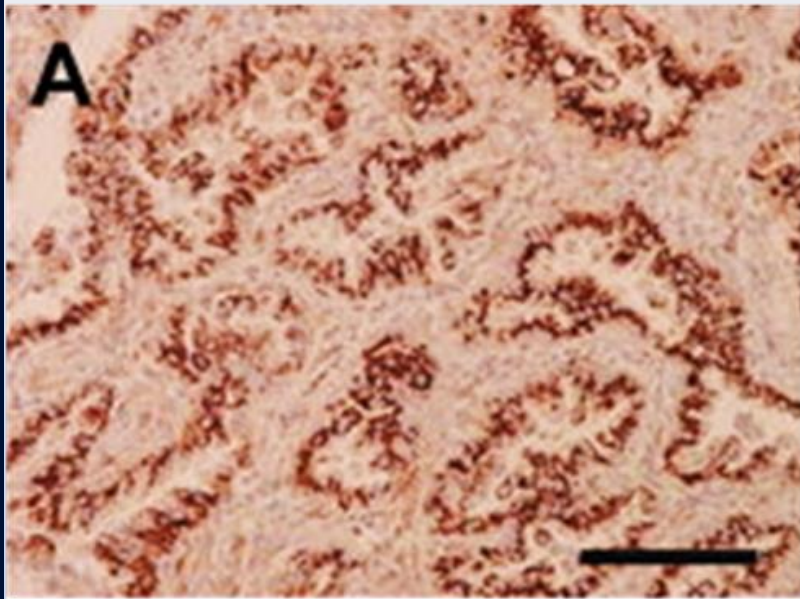
Normal Breast Duct



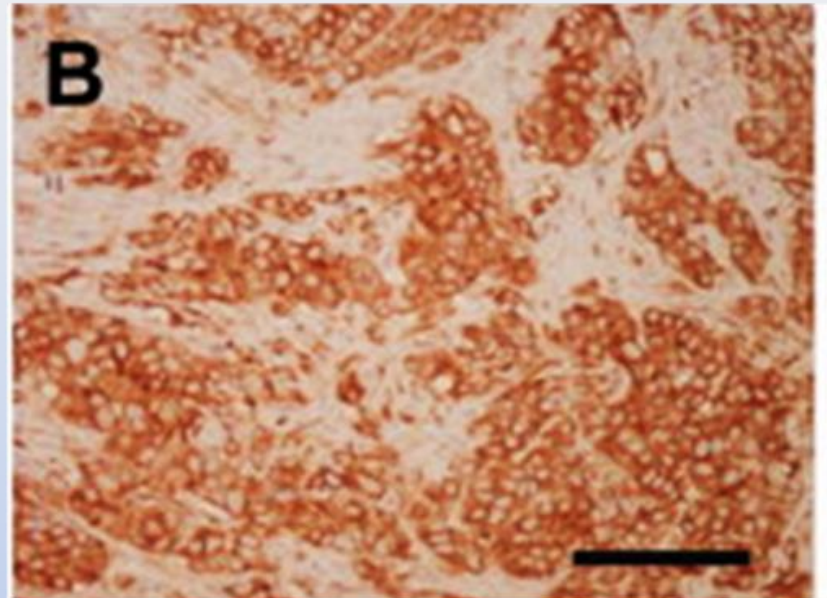
Lactating Breast

β V-tubulin in Malignancies

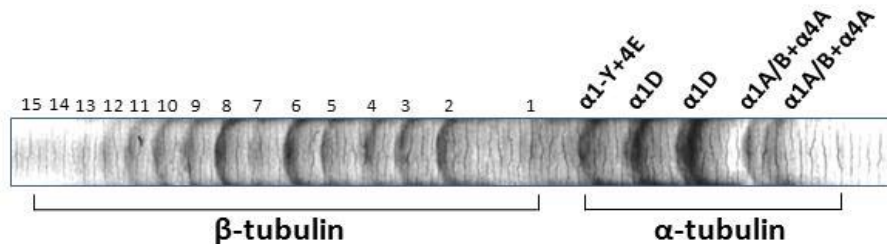
Lung Adenocarcinoma



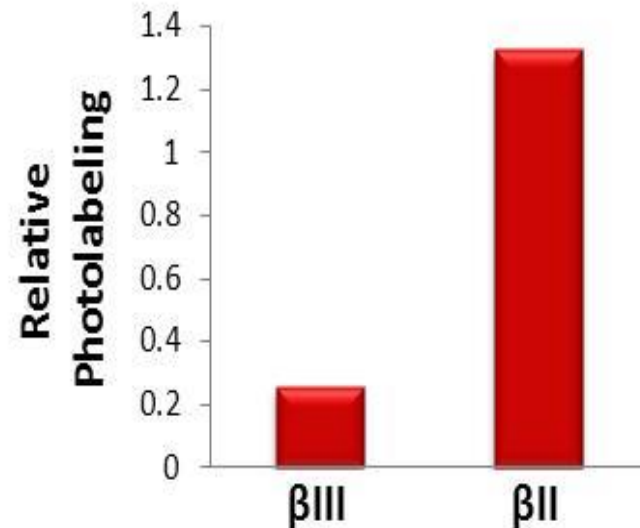
Invasive Breast Cancer



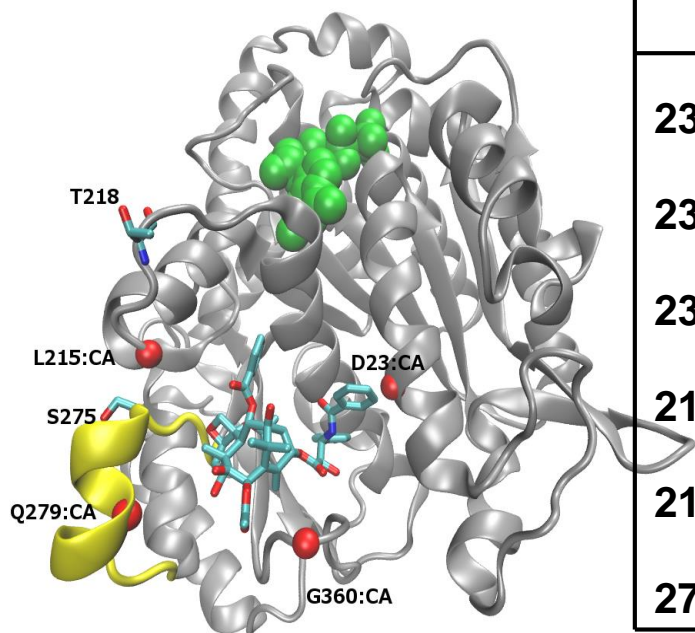
Photolabeling of Tubulin Isoforms with [³H]2-(m-azidobenzoyl)taxol



Band #	Tubulin Isotype	% Total β-tubulin	% Total Photolabeling
1	βIII	6.7	1.7
2	βIVb	9.1	2.1
3	βIVb	8.2	2.0
4	βI + βII + βIVa	8.4	6.3
5	βIVa	8.2	8.4
6	βII + βIVb	11.2	5.9
7	βII (+3E)	6.7	11.2
8	βII (+4E)	11.4	9.3
9	βII (+4E)	8.8	9.1
10	βII (+4E)	8.2	12.3
11	βII (+4E)	7.1	8.5
12	βII (+5E)	3.7	6.3
13	βII (+6E)	1.3	5.9
14	βII (+ nE)	1.0	6.2
15	βII (+ nE)	--	4.7



Distances Across Binding Pocket in Monomers of β I-Tubulin and T218A Variant



Distance definition	Distance in Taxol complex (Å)	β I-Tubulin Av distance (Å) *	T218A Variant Av distance (Å) *
23-215	18.39	20.1 97.3	18.9 63.5
23-279	23.34	26.5 99.2	23.1 51.5
23-360	11.34	12.4 73.3	12.0 67.2
215-279	9.8	11.3 95.2	12.8 85.3
215-360	16.43	17.6 80.3	17.5 78.7
279-360	16.06	18.5 90.7	14.7 36.3

- % of snapshots with distance \geq distance in Taxol complex
Molecular dynamic simulations – frequency of Taxol-accommodating conformations decreased in the variant.

Conclusions

Tumors from different origins express distinct tubulin isotypes.

Taxol binds differentially to distinct β -tubulin isotypes.

Tubulin isotypes have a role in the response of tumors to Taxol and other antitumor drugs. Therefore, knowing the isotype content of tumors may affect treatment in a positive way.

Tubulin isotypes may have effects in cells unrelated to their role as components of the microtubule cytoskeleton.

Acknowledgements



Albert Einstein College of Medicine

Suzan Chao
Meagan Vogt
Jing Hu
Marina Khrapunovich-Baine
Deepti Mathew
Vilas Menon
Leah Miller
Graciela Rodriquez
Leleesha Samaraweera
Eng-Hui Yap
Pascal Verdier- Pinard

Ruth Angeletti
Andras Fiser
Gloria Huang
Hayley McDaid
Hui Xiao
Chia Ping H. Yang
Yanhua Wang

